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REVIEW ARTICLE

New Targetable Oncogenes in Non–Small-Cell Lung Cancer

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ABSTRACT

The identification of oncogenic driver mutations underlying sensitivity to epidermal growth factor receptor and anaplastic lymphoma kinase tyrosine kinase inhibitors has led to a surge of interest in identifying additional targetable oncogenes in non–small-cell lung cancer. A number of new potentially oncogenic gene alterations have been characterized in recent years, including *BRAF* mutations, *HER2* insertions, *PIK3CA* mutations, *FGFR1* amplifications, *DDR2* mutations, *ROS1* rearrangements, and *RET* rearrangements. In this review, we will discuss the techniques used to discover each of these candidate oncogenes, the prevalence of each in non–small-cell lung cancer, the preclinical data supporting their role in lung cancer, and data on small molecular inhibitors in development.

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INTRODUCTION

The treatment of non–small-cell lung cancer (NSCLC) has been transformed by the identification of rationally targeted therapies for a subset of molecularly defined lung cancers. Initial experience with targeted therapies for unselected patients with NSCLC resulted in modest and inconsistent improvements in outcomes. Although the addition of bevacizumab to first-line doublet chemotherapy improved median survival by 2 months in one randomized study, it failed to improve survival in another.[1-3] Treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in unselected patients with NSCLC resulted in similarly modest effects: Erlotinib in previously treated NSCLC improved median survival by 2 months,[4] whereas gefitinib did not significantly improve survival in a similar population.[5]

In contrast, treatment of molecularly selected populations with rationally targeted therapies has led to unprecedented results. Prospective studies of erlotinib and gefitinib in patients with *EGFR*-mutant lung cancer have resulted in response rates greater than 60%, and randomized trials in both Asia and Europe have shown a marked improvement in progression-free survival (PFS) over first-line chemotherapy, albeit with no overall survival benefit because of patient crossover.[6,7] Initial data with crizotinib in *ALK*-rearranged lung cancer have demonstrated similar response rates[8]; studies randomly assigning patients to crizotinib versus standard chemotherapy are under way.[8a] The remarkable effectiveness of these drugs in molecularly selected populations has led to a surge in the number of lung cancer trials studying targeted therapies in other molecularly selected populations.

In this review, we will discuss emerging data on new targetable oncogenes in NSCLC. We will defer to other reviews in this series for a discussion of cancers with *KRAS* mutations and *MET* alterations. We will focus on genes encoding protein kinases, given the important recent progress in treating lung cancer through the use of targeted kinase inhibitors. The emerging therapies directed at immune modulation, apoptosis, and chaperone proteins are outside of the spectrum of this review. Although several of these new oncogenes are found rarely in NSCLC, in total they constitute 9% to 14% of lung adenocarcinomas (Table 1) and 16% to 30% of squamous cell lung carcinomas (Table 2; Fig 1). To highlight the varied strategies that have been used in recent years to identify new targetable oncogenes, this review will discuss oncogenes grouped by the technique in which they were identified (Fig 2).

ONCOGENIC ALTERATIONS IDENTIFIED THROUGH FOCUSED ANALYSIS OF KNOWN PROTO-ONCOGENE

The most straightforward approach toward the identification of driver mutations in NSCLC has been to search for alterations in known proto-oncogenes, coding proteins with an established important role in cellular growth signaling. Through this approach, several new molecular targets in NSCLC have been identified, including *HER2* insertions, *BRAF* mutations, and *PIK3CA* mutations.

Table 1. New Targetable Oncogenes in Lung Adenocarcinoma

Oncogenic Target	Prevalence (%)	Reported Clinical Associations	Potential Kinase Inhibitors
HER2 insertions	2.8[9]	Never-smokers[9] Asian race[9] Female sex[9]	Afatinib (BIBW-2992) Neratinib (HKI-272) Dacomitinib (PF-00299804)
BRAF mutations	2 to 4.9[10-12]	Ever-smokers[10] White race[10] V600E: never-smokers[11] V600E: female sex[11]	Vemurafenib GSK2118436
PIK3CA mutations	1.5 to 2.6[12-15]	No association seen[13-15]	GDC-0941 XL147 BKM120
RET rearrangements	1.2[16] 1.9[17]*	Never-smokers[16] Asian race[17]	Vandetanib Sorafenib Sunitinib Cabozantinib (XL184)
ROS1 rearrangements	1.2 to 2.6[16,18]	Never-smokers[18] Asian race[18] Younger age[18]	Crizotinib

*Never-smokers or former limited smokers.

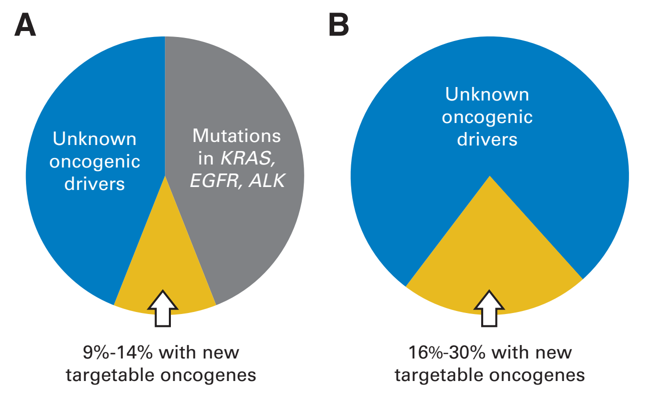

Fig 1. Prevalence of new targetable oncogenes in non–small-cell lung cancer. Although several of the individual oncogenic alterations are relatively rare, in total they constitute (A) 9% to 14% of lung adenocarcinomas and (B) 16% to 30% of squamous cell lung carcinomas.

HER2 *Insertions*

Human epidermal growth factor receptor 2 (HER2, or ERBB2) is a membrane-bound tyrosine kinase in the ERBB family; unlike EGFR (or EBRR1), HER2 has no known ligand but will heterodimerize with other members of the ERBB family on ligand binding. The *HER2* gene has been a recognized proto-oncogene in human cancers for more than two decades, since it was found to be amplified in approximately 30% of breast cancers. HER2-positive breast cancer has historically been associated with a poorer prognosis, although outcomes are improved significantly through the use of HER2-targeted agents like trastuzumab.[23,24] HER2 has also been found to be amplified or overexpressed in a subset of gastric carcinomas, in which it is associated with improved outcomes through the addition of trastuzumab to chemotherapy.[25] Although HER2 is also overexpressed in 13% to 20% of NSCLCs (with 3+ expression in only 2% to 6%),[26-29] a study of trastuzumab in HER2-overexpressing NSCLC did not identify significant clinical activity.[30] *HER2* amplification on fluorescent in situ hybridization (FISH) is seen in approximately 2% to 4% of NSCLCs (more commonly in adenocarcinomas),[26,27] but trastuzumab sensitivity in this population has not been clearly studied.

Table 2. New Targetable Oncogenes in Squamous Cell Lung Carcinoma

Oncogenic Target	Prevalence	Reported Clinical Associations	Potential Kinase Inhibitors
PIK3CA mutations	3.6 to 6.5[13,14,19]	No association seen[13,14]	GDC-0941 XL147 BKM120
FGFR1 amplification	9.7 to 21[20,21]	Ever-smokers[20]	Brivanib (BMS-582664) Dovitinib (TKI258) Ponatinib (AP24534) E3810
DDR2 mutations	2.2[22]	No association seen[22]	Dasatinib Imatinib Nilotinib

The initial identification of *EGFR* mutations in a subset of lung adenocarcinomas led to subsequent interest in identifying such mutations in *HER2*, particularly given the homology between these two ERBB-family kinases. In the initial report, 120 patients with NSCLC underwent *HER2* sequencing; mutations were identified in five (4.2%) and were found to be nonoverlapping with *KRAS* and *EGFR* mutations.[31] A second study of 671 patients with resected NSCLC found *HER2* mutations in 11 (1.2%); all occurred in those with adenocarcinoma, where the prevalence was 2.8%.[9] All of the mutations identified were in-frame insertions of three to 12 base pairs into exon 20 and were found to be more prevalent in never-smokers. *HER2* insertions have been found to induce constitutive activation of the HER2 kinase in a ligand-independent fashion,[32] similar to the effect of *EGFR* mutations. Indeed, parallel exon 20 insertion mutations in the *EGFR* gene can be identified with a similar frequency in NSCLC, in approximately 1.9% of patient cases.[33] Inducible tissue-specific overexpression of mutant *HER2* in a transgenic mouse model was found to result in rapid development of NSCLC, confirming the oncogenicity of this driver mutation.[34]

Several kinase inhibitors are in development for *HER2*-dependant lung adenocarcinoma. The most promising compounds are irreversible TKIs targeting HER2 and EGFR, such as neratinib (HKI-272), dacomitinib (PF-00299804), and afatinib (BIBW-2992).[35] Neratinib and dacomitinib have both been found to effectively inhibit the growth of *HER2*-mutant lung cancer cell lines as well as cell lines transformed by the introduction of HER2.[32,36] Afatinib was found to induce modest regressions when introduced into the previously mentioned transgenic mouse model, and this effect was potentiated by the addition of rapamycin, a mammalian target of rapamycin (mTOR) inhibitor, suggesting a particular dependence on the Akt/mTOR pathway in *HER2*-mutant lung cancer.[34] Although there are limited data regarding mTOR inhibitors in patients with *HER2*-mutant lung adenocarcinoma, a phase I trial of neratinib plus temsirolimus resulted in partial responses in two of six patients with *HER2*-mutant NSCLC.[37] An early report from a phase II trial of afatinib alone in molecularly selected lung adenocarcinoma

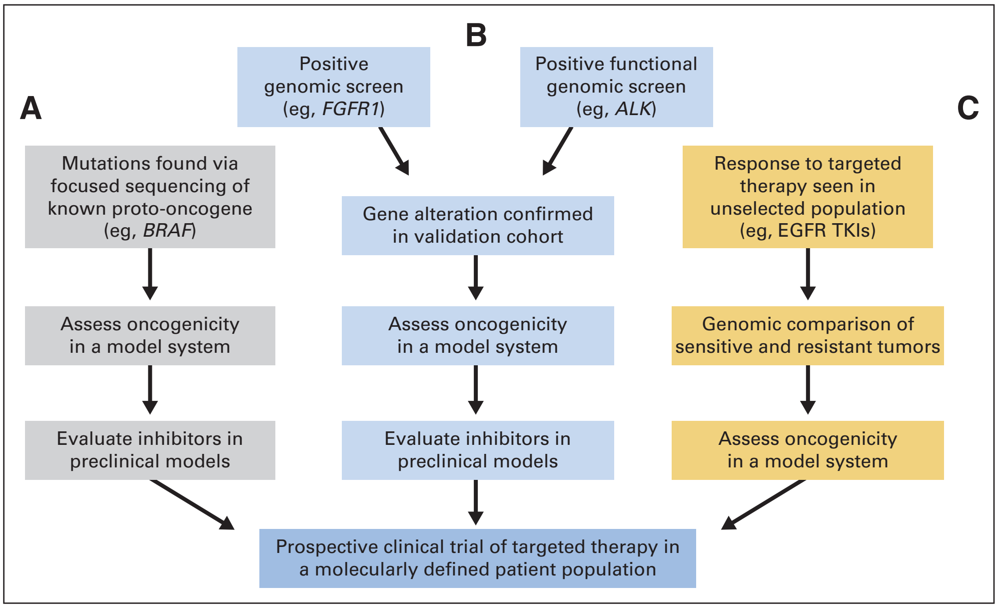

Fig 2. Strategies used to identify targetable oncogenes in non–small-cell lung cancer (NSCLC). (A) Alterations in recognized proto-oncogenes (eg, *BRAF* mutations) can be identified through focused sequencing efforts. (B) Unexpected oncogenes (eg, *FGFR1* or *ALK*) can be discovered through genome-wide screening strategies, with the requirement that initial positive results are subsequently confirmed in a separate cohort of clinical specimens. (C) Analysis of specimens from a trial of a targeted therapy (eg, epidermal growth factor receptor [EGFR] tyrosine kinase inhibitors [TKIs]) has the advantage of being inherently tied to an available treatment, although there may be limitations to what genomic analyses are feasible on biopsies from patients with advanced NSCLC. All candidate oncogenes should demonstrate oncogenicity in a model system before they can be considered driver mutations.

(NCT00730925) describes partial responses in two of five patients with tumors harboring *HER2* mutations[38]; efficacy may have been limited by toxicity, which required several patients to come off study before progression. Interestingly, *HER2*-amplified NSCLC may also gain benefit from HER2 kinase inhibitors, based on a report of a marked response seen in one patient receiving dacomitinib.[39] However, a prospective trial of dacomitinib has now reported only three responses out of 18 *HER2*-mutant lung cancers,[39a] suggesting that dacomitinib alone may be inadequate to fully inhibit this particular oncogene in patients.

BRAF *Mutations*

BRAF is a serine/threonine kinase downstream from KRAS in the mitogen-activated protein kinase (MAPK) signaling cascade. Although *KRAS* mutations have been well established in human cancer for several decades, *BRAF* mutations were only first identified in 2002, with a particularly high prevalence identified in melanoma.[40] *BRAF* mutations were found most commonly in exon 15, resulting in a substitution of a glutamine for a valine at residue 600 (V600E); this substitution is believed to destabilize the inactive conformation of the kinase, resulting in constitutive kinase activation and downstream phosphorylation.[41] *BRAF* mutations have since been identified in a variety of human cancers, including colorectal cancer,[42] papillary thyroid cancer,[43] and hairy cell leukemia.[44]

The high incidence of oncogenic *BRAF* mutations in melanoma led investigators to search for *BRAF* mutations in NSCLC. In two initial reports, each studying more than 100 patient cases of NSCLC, sequencing of exons 11 and 15 of *BRAF* identified missense mutations in 1.6% and 3%, respectively; a majority of the mutations were non-V600E.[45,46] More recently, 697 and 739 lung adenocarcinomas were genotyped by two groups of US and Italian investigators, identifying missense mutations in 3% and 4.9% of patient cases, respectively[10,11]; the lower prevalence in one study may have been the result of a focal genotyping technique looking only at codons V600, D594, and G469.[10] Approximately half of the mutations identified were V600E, with one group finding that this particular mutation was more common in never-smokers.[11] Importantly, non-V600E mutations seem to be relatively more common in NSCLC than in colorectal cancer or melanoma and were found to occur exclusively in patients with lung cancer with a smoking history.

The potential role of *BRAF* V600E as targetable driver mutation in lung adenocarcinoma is strengthened by the finding that in vivo expression of V600E in mouse models leads to development of invasive adenocarcinoma, a phenotype that is reversed when V600E expression is stopped.[47] This finding, which parallels the behavior of *EGFR*-activating mutations in mouse models, fuels optimism that *BRAF*-mutant lung adenocarcinoma may be highly sensitive to TKIs like vemurafenib, which has demonstrated potent anticancer activity in melanomas harboring *BRAF* V600E mutations.[48] However, this enthusiasm must be tempered by the experience in colon cancer, in which BRAF inhibitors have been found to have little activity against tumors carrying V600E mutations.[49] The contrasting phenotypes seen in these two types of *BRAF*-mutant cancer suggest that genotype alone may be inadequate for the prediction of sensitivity to targeted therapy; a better understanding of tissue-specific factors modulating oncogenic sensitivity to TKIs is needed. One instance of a positron emission tomography response to vemurafenib in a patient with NSCLC harboring *BRAF* V600E has been reported,[50] and a phase II trial of the BRAF inhibitor GSK2118436 is currently under way in *BRAF* V600E–mutant NSCLC (NCT01336634) to prospectively study this approach in lung cancer. Because cancers with non-V600E *BRAF* mutations are unlikely to be inhibited by V600E-specific inhibitors, inhibitors of downstream targets (ie, MEK) are being explored in this population (NCT00888134) because these have be found to be active in *BRAF*-mutant melanoma.[51]

PIK3CA *Mutations*

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that play an important role in regulating cell growth, proliferation, and survival. Mutations in PI3Ks were first identified through a sequencing analysis of eight PI3K genes in 297 cancer specimens (primarily colon cancers).[52] Point mutations in the *PIK3CA* gene, encoding the catalytic subunit of the class 1 PI3Kα, were found in several cancer types, including one of 24 lung cancer specimens studied. In three subsequent large analyses of 229, 235, and 691 NSCLC specimens, *PIK3CA* mutations were found in three (1.3%), eight (3.4%), and 11 (1.6%) patient cases, respectively,[13,14,53] with prevalence being higher in squamous cell carcinoma. A recent study genotyped 95 resected squamous cell lung cancers (all p63+/TTF1−), and *PIK3CA* mutations were found in four (4%), whereas no mutations in *EGFR*, *KRAS*, *ALK*, *HER2*, or *BRAF* were identified.[19]

An important finding has been that *PIK3CA* mutations in lung adenocarcinoma often coexist with another oncogenic mutation, such as an *EGFR* or *KRAS* mutation,[13,15] raising suspicion that this may be a secondary process and not a true driver mutation. Yet genetically engineered mouse models have demonstrated that expression of mutant *PIK3CA* on its own can induce multifocal adenocarcinoma, and these tumors rapidly regress when expression ceases.[54] In lung cancer cell lines with *PIK3CA* mutations, it has also been shown that PI3K activity is elevated, and RNAi knockdown of PI3K causes growth arrest.[14] One could hypothesize that a portion of *PIK3CA*-mutant lung cancers are dependent on this driver oncogene, whereas in other patient cases, the mutation may modulate the effect of another oncogenic process.

Multiple PI3K inhibitors are in development, although it remains uncertain what population should be targeted with these agents. In the previously described genetically engineered mouse model of *PIK3CA*-mutant lung cancer, a PI3K inhibitor with broad activity was found to inhibit PI3K phosphorylation and induce tumor response.[54] However, there is debate in the breast cancer literature as to whether PI3K inhibitor activity is truly limited to those cancers with *PIK3CA* mutations,[55] particularly given the broader activity seen with mTOR inhibitors. Ongoing trials in lung cancer include single-agent PI3K inhibitors (NCT01501604) as well as combinations with chemotherapy (NCT00974584, NCT00756847) and other targeted agents (NCT00974584).

ONCOGENIC ALTERATIONS IDENTIFIED THROUGH GENOMIC SCREENING STRATEGIES

Genomics is a discipline with a broad interest in describing the landscape of genetic alterations in a given organism or disease. Although this discipline was not specifically developed for the purpose of identifying targetable oncogenes, genomic tools can be used to screen broadly for genetic alterations of potential interest in cancer treatment. Cancer genomics primarily studies structural DNA alterations—missense mutations, copy number alterations, and translocations.[56] There are a wide variety of genomic analysis technologies available, each with specific strengths and weaknesses that have been recently reviewed in detail.[57] Potentially targetable alterations identified using these techniques include *FGFR1* amplifications, *DDR2* mutations, and *RET* rearrangements.

FGFR1 *Amplification*

The important advances achieved over the past decade through identifying oncogenic mutations in lung adenocarcinoma have led to several efforts to screen for targetable oncogenes in squamous cell carcinoma of the lung. An initial screen of 155 squamous cell lung cancer specimens using single nucleotide polymorphism arrays identified focal amplifications of the *FGFR1* gene.[20] FGFR1 is a membrane-bound receptor tyrosine kinase that regulates proliferation via the MAPK and PI3K pathways, much like EGFR. High-level amplification of *FGFR1* (chromosome 8p) was seen in 9.7% of patient cases in addition to the previously described amplification of *SOX2* (chromosome 3q).[58] Seventy-seven lung adenocarcinoma specimens were also studied, and *FGFR1* amplification was seen in only 1%. A second analysis has confirmed the finding that *FGFR1* amplification is much more common in squamous cell lung cancers (21%) than in lung adenocarcinoma (3.4%).[21] Survival of *FGFR1*-amplified lung cancer cell lines was additionally shown to be dependent on overexpression of the FGFR1 kinase.[21,58]

Several small molecular inhibitors of FGFR1 are in development, and initial studies indicate activity against *FGFR1*-amplified lung cancer models. The FGFR inhibitor PD173074 (with activity against multiple FGFR-family kinases as well as vascular endothelial growth factor receptor 2 [VEGFR2][59]) has been found to inhibit the growth of *FGFR*-amplified lung cancer cell lines[21,58] and also led to responses in mouse xenografts.[58] Sensitivity to PD173074 was not seen in cell lines without *FGFR1* amplification. Clinically, the FGFR inhibitor farthest along in development is brivanib (BMS-582664), a dual inhibitor of FGFR and VEGFR signaling with an adverse effect profile similar to those of other VEGF inhibitors.[60] In a randomized discontinuation study of brivanib in 396 patients with five types of advanced solid tumors, no partial responses were seen in 42 patients with previously treated NSCLC.[61] A second generation of FGFR inhibitors with greater specificity (and fewer toxicities) is in development.[62]

DDR2 *Mutations*

DDR2 is a membrane-bound receptor tyrosine kinase that binds to collagen and can regulate proliferation and migration. Mutations in the *DDR2* gene were recently identified in squamous cell lung cancer through a sequencing screen of 201 genes with a potential role in human cancer (including the entire tyrosine kinome).[22] Investigators used an iterative approach for this analysis: In an initial screen, 20 tumors were sequenced, and mutations were identified in six tyrosine kinase genes, including *DDR2*. In a secondary screen of 48 tumors and cell lines, sequencing of just the six candidate tyrosine kinase genes was performed, and four *DDR2* mutations were found. Lastly, a validation cohort of 222 specimens underwent sequencing of *DDR2* alone, and mutations were found in five patient cases (2.2%). Supporting the oncogenic role of *DDR2*, the investigators showed that *DDR2*-mutant cell lines had impaired proliferation through shRNA knockdown of DDR2 expression. It should be noted that a prior genotyping effort found no *DDR2* mutations in 54 squamous cell lung cancers,[63] but these investigators only tested for a single mutation (R105S), which was not one of the mutations identified in the larger screening study.

Although DDR2 has not been a major focus of drug development efforts, a recent analysis showed that ABL kinase inhibitors such as imatinib, nilotinib, and dasatinib have activity against DDR2.[64] In the previously mentioned study, it was found that dasatinib inhibited the growth of *DDR2*-mutant lung cancer cell lines and caused regressions of *DDR2*-mutant mouse xenographs. Unfortunately, dasatinib has not been found to be especially active against NSCLC, with only one partial response seen in a phase II study of 34 patients,[65] although only six of the patients in this study had squamous cell carcinoma. Intriguingly, a patient with squamous cell lung cancer responding to dasatinib and erlotinib in a separate phase II study[66] had tissue available for

DDR2 sequencing, and a point mutation in the *DDR2* kinase domain was identified.[22]

There is optimism that *DDR2* mutations will prove to be the first targetable mutations in squamous cell lung carcinoma; however, there remain a few unresolved questions. For example, although the initial screening study identified 11 total mutations in *DDR2*,[22] these mutations occurred at 10 different codons (with only five mutations in the kinase domain) rather than there being a few recurring mutations. Whether all of these variants are oncogenic and sensitive to dasatinib will need to be determined. Additionally, an important toxicity of dasatinib therapy is the development of pleural effusions, a challenging adverse effect for many patients with lung cancer, who already have limited pulmonary reserve. To clarify the feasibility of this approach, two phase II trials of dasatinib in squamous cell lung carcinoma are under way (NCT01491633, NCT01514864).

RET *Rearrangements*

RET is a receptor tyrosine kinase with a well-established role in human cancer: Missense mutations in *RET* are common in sporadic medullary thyroid cancer, and *RET* rearrangements can be identified in a subset of papillary thyroid cancers.[67] *RET* rearrangements were recently identified in a subset of lung adenocarcinomas by several groups using different screening strategies. Two groups performed whole-transcriptome sequencing of RNA extracted from lung adenocarcinoma specimens, and each identified a patient case with an unexpected fusion between *KIF5B* and *RET*, two genes separated by more than 10 Mb on chromosome 10.[68,69] This same fusion gene was identified by a third group through use of targeted next-generation sequencing of 2,574 exons and 37 introns from 152 genes with importance in cancer pathways.[17] Introns from *RET* were included in the sequencing effort because of the role of *RET* rearrangements in thyroid cancer, leading to the identification of *KIF5B-RET* in one of 24 NSCLC specimens studied. Lastly, a fourth group identified the *KIF5B-RET* fusion through a FISH screen for new fusions involving *KIF5B*,[16] given their previous identification of a rare *ALK* rearrangement involving *KIF5B*.[70] Using a subsequent FISH screen for new *RET* fusions, this fourth effort additionally identified an alternate fusion partner for *RET*, the *CCDC6* gene. It was additionally confirmed that *KIF5B-RET* was oncogenic when expressed in cell lines or implanted into xenographs.[16,17,69] In the largest prevalence analysis, combined *RET* FISH and real-time polymerase chain reaction (RT-PCR) identified 13 tumors (1.2%) with *RET* rearrangements among 1,119 lung adenocarcinomas.[16] In an alternate prevalence analysis of 159 lung adenocarcinomas from never-smokers or former light smokers, who were wild type for know oncogenic alterations (in *EGFR*, *KRAS*, *ALK*, *HER2*, *BRAF*, and *ROS1*), 10 *RET* rearrangements (6.3%) were identified.[17]

Several commercially available TKIs have activity against the RET kinase. Vandetanib, initially developed as an *EGFR* inhibitor, has now been US Food and Drug Administration (FDA) approved for the treatment of advanced medullary thyroid carcinoma based on an improvement in PFS and response rate over placebo.[71] Vandetanib has been tested in several cell lines transformed by *KIF5B-RET* and was found to inhibit RET phosphorylation and suppress cell growth.[16,17,69] Sunitinib and sorafenib, two VEGFR inhibitors with activity against the RET kinase, have also been found to inhibit the growth of *KIF5B-RET*–transformed cell lines.[17] Cabozantinib is a new kinase inhibitor with activity against medullary thyroid cancer that also has activity against *KIF5B-RET*–transformed cell lines.[72] Each of these multikinase inhibitors has been found to have some degree of activity in NSCLC previously, but it remains to be determined whether sensitivity to these drugs in patients with lung cancer is the result of underlying *RET* rearrangements.

ONCOGENIC ALTERATIONS IDENTIFIED THROUGH FUNCTIONAL GENOMIC SCREENING STRATEGIES

Functional screening looks beyond the structure of the cancer genome to identify genes or proteins specifically associated with oncogenicity. *ALK* rearrangements were first identified in this fashion.[73] Using a retrovirus, coding DNA sequences were generated from RNA extracted from a lung adenocarcinoma specimen. These DNA sequences were transfected into cells that would only grow if the DNA sequence were sufficiently oncogenic and coded for a protein that could sustain cellular growth. Through study of one of these functionally selected DNA sequences, a fusion protein of *EML4* and *ALK* was identified and subsequently studied. Because of their greater specificity for the identification of oncogenic alterations, there are a number of such functional screening assays now being used, one of which led to the identification of *ROS1* rearrangements in NSCLC.

ROS1 *Rearrangements*

ROS1 is a receptor tyrosine kinase with homology to the insulin receptor and signals down the MAPK signaling cascade through phosphorylation of RAS. The *ROS1* gene was first identified as an oncogene in NSCLC through a phosphoproteomic screen, leveraging the fact that oncogenic kinases should be highly phosphorylated proteins.[74] Studying 41 NSCLC cell lines and 150 tumors, investigators examined which kinases had elevated activity when compared with the average activity of that kinase across all specimens. Several expected oncogenes were identified among the highly phosphorylated kinases, including EGFR, HER2, and MET. They additionally identified that ALK, ROS1, and PDGFRα were highly phosphorylated, both in some cell lines and some tumor specimens. Studying RNA transcripts from these specimens using RT-PCR, several unexpected fusion proteins were identified, including two *ALK* fusions and two *ROS1* fusions (*SLC34A2-ROS1* and *CD74-ROS1*). Whereas *ROS1* rearrangements had previously been identified in glioblastoma,[75] this was the first description of *ROS1* rearrangements in NSCLC.

The prevalence of *ROS1* rearrangements was recently studied in a multi-institutional series of 1,073 patients with NSCLC using a break-apart FISH assay.[18] Of the 694 adenocarcinomas studied, 18 tumors (2.6%) were positive for *ROS1* rearrangements by FISH, although translocations could be confirmed by RT-PCR in only six patients. Rearrangements were found to be more common in never-smokers and Asian patients and were associated with younger age. In a subsequent FISH analysis of 1,116 patients with lung adenocarcinoma, 13 (1.2%) had rearrangements, and 11 of these were confirmed by RT-PCR.[16] This second study identified three new fusion partners for *ROS1*: *TPM3*, *SDC4*, and *EZR*.

ROS1 has been a recognized oncogene in glioblastoma for many years,[75] but selective ROS1 inhibitors have not yet been developed clinically. However, it has been identified that cells carrying ROS1 rearrangements have sensitivity to ALK inhibitors. Using an automated platform, investigators screened 602 cell lines for sensitivity to

TAE684, a selective ALK inhibitor, and found that 10 cell lines had greater than 50% cell kill after 72 hours of treatment.[76] Although most of these cell lines harbored ALK alterations, one cell line (HCC-78) had previously been found to harbor a *ROS1* translocation.[74] After confirming that this cell line was similarly sensitive to crizotinib, investigators treated a patient with *ROS1*-rearranged lung adenocarcinoma with crizotinib, and he had a near complete response.[18] Fourteen patients with *ROS1*-rearranged lung cancers have now been treated in an expansion cohort of the ongoing phase I trial of crizotinib, and nine (64%) had a confirmed response.[77]

ONCOGENIC ALTERATIONS IDENTIFIED THROUGH STUDY OF CANCERS SENSITIVE TO TARGETED THERAPY

One final strategy for oncogene discovery is the strategy initially used to identify *EGFR* mutations in NSCLC. With this strategy, a therapy with known targets is administered to an unselected patient population, and subsequent analysis of tissue from responders can potentially identify an oncogenic alteration underlying sensitivity. This is an extremely attractive strategy for oncogene discovery because it is inherently tied to a treatment option. However, this strategy can be quite challenging—years after FDA approval, there is still no biomarker that clearly predicts for bevacizumab sensitivity.[78] In the case of EGFR-sensitizing mutations, this oncogene was easier to identify for several reasons: first, EGFR was known to be commonly overexpressed and was a suspected oncogene; second, *EGFR* mutations are relatively common compared with other alterations; and third, *EGFR* mutations can be identified with a relatively simple technology (ie, Sanger sequencing).

Going forward, several important challenges will be encountered when using prospective clinical trials to identify oncogenic alterations underlying sensitivity. Most important is tissue adequacy, both in terms of quantity and quality. A core biopsy will be needed for most genomic analysis, but those requiring more than a core biopsy may be difficult to perform consistently in a cohort of patients with advanced NSCLC. Some analysis techniques may require fresh/frozen tissue, such that a fresh biopsy will need to be obtained on study entry. After the molecular characteristics of the tumors have been determined, the analysis will require that patients be separated into sensitive and resistant groups in some meaningful fashion. How to identify the sensitive phenotype using conventional computed tomography imaging is somewhat unclear; some have found that minor response may correlate more closely with presence of an underlying driver mutation than RECIST partial response.[79]

DISCUSSION

In conclusion, steady progress is being made in identifying targetable oncogenes in lung adenocarcinoma and squamous cell carcinoma. Although many of these oncogenic alterations are uncommon, rationally targeted therapies can have a profound effect on outcomes in these subpopulations of NSCLC. At present, we recommend testing for these genomic alterations if feasible to determine clinical trial eligibility for appropriate patients.

A key challenge in the coming years will be developing efficient assays for identifying these genomic alterations such that appropriate targeted therapies can be delivered. Because sequential testing for mutations in each oncogene may be impractical, personalized cancer care is likely to eventually require multigene assays such as those reviewed elsewhere in this series. To facilitate the identification of new oncogenes underlying drug sensitivity, the greatest progress will be made by ensuring that prospective trials of targeted therapies are accompanied by painstaking collection of tumor tissue.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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